Theoretical Studies on β-Lactam Antibiotics. IV. Conformational Analysis of Novel β-Lactam Antibiotics and the Binding Specificities of Crosslinking Enzyme(s) and β-Lactamases

T. K. VASUDEVAN and V. S. R. RAO, *Molecular Biophysics Unit, Indian Institute of Science, Bangalore-560 012, India*

Synopsis

Conformational-energy calculations were carried out on the new family of β-lactam antibiotics (viz., thienamycin, PS-5, 1-oxa- and 1-thiapenems, and their close analogs); these exhibit broad-spectrum antibacterial activity and stability towards β-lactamase-producing strains. The bicyclic ring system in all the compounds studied was found to be highly rigid and to favor only one conformation. This is in contrast to findings in penicillins, where the five-membered ring assumes two puckered conformations. The relative orientations of the bicyclic ring system and the nature and configuration of the substituent at C-5 position, besides nonplanarity of the lactam peptide bond, are shown to be important for biological activity. The present study, in agreement with x-ray studies, predicts that the lactam peptide bond in 1-carbapenem is more nonplanar than in 1-thiapenem. These studies also suggest that the conformational requirement of bicyclic ring system to bind to crosslinking enzyme(s) and β-lactamases is very similar.

INTRODUCTION

The β-lactam antibiotics, which inhibit the growth of many gram-positive and gram-negative bacteria, exert their lethal action by interfering with the bacterial cell wall biosynthesis.[1,2] Depending on the organism and the antibiotic, the inhibition can involve a transpeptidase or a carboxypeptidase or both and leads either directly or indirectly to the death of growing bacteria.[1-5] Tipper and Strominger proposed that β-lactam antibiotics derive their antibacterial activity because of their structural analogy to the terminal D-alanyl-D-alanine of nascent pentapeptide side chains of peptidoglycans.[6] Recent theoretical studies of Virudachalam and Rao[7,8] have shown that such an analogy exists between penicillin and terminal D-alanyl-D-alanine, and this is due to the presence of lactam ring in the antibiotic. These studies have also indicated that the specific orientations of the amino acyl and carboxyl groups are also important in addition to the nonplanarity of the lactam peptide bond.

Penicillin is one of the most potent and widely used antibiotics in combating bacterial diseases. However, its widespread use has led to the emergence of resistant bacteria which produce β-lactamases that inactivate

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penicillins. Hence, there is a constant need for designing better drugs that are active against the resistant strains.

Recently, significant advances have been made both in the synthesis and isolation of potent novel β-lactam antibiotics which are also active against resistant strains.[9–24] Though the new antibiotics have some common features, there are distinct differences in the position of double bond and in the nature or configuration of the groups at different places of the antibiotics. Such differences may affect the nonplanarity of the lactam peptide bond and the orientations of the carboxyl group and the side group at C-6 differently, and these may account for their differences in activity. Therefore, a detailed analysis of the conformational flexibility of these molecules has been carried out by semiempirical potential-energy calculations, and the results are presented here. These studies may throw light on the binding requirements of crosslinking enzyme(s) and β-lactamases, knowledge of which is essential in the design of new drugs.

METHOD OF CALCULATION

Nomenclature

The numbering of atoms and dihedral angles in these molecules is indicated in Fig. 1. (See Figs. 2–4 for the molecules.) The dihedral angles α_1, α_2, and α_3 specify the relative orientations of the planes (5-1-2) and (2-3-4) and of the mean plane through (4-5-6-7), respectively, with respect to the reference plane (2-4-5). Hence, in this system, α_1 and α_2 define the conformations of the five-membered ring and α_3 defines its orientation with respect to the four-membered ring. Clockwise rotation about the virtual or real bonds (indicated by arrows) looking along (5-2), (2-4), and (4-5) is considered as positive for the dihedral angles α_1, α_2, and α_3. The dihedral angles ϕ, ψ, and ω are defined as follows:

$\phi_1 = 0$ when bond (6-7) eclipses the bond (X_3-X_4)
$\psi_1 = 0$ when bond (7-4) eclipses the bond (6-X_3)
$\omega = 0$ when bond (4-3) eclipses the bond (7-6)
$\phi_2 = 0$ when bond (3-9) eclipses the bond (4-7)
$\psi_2 = 0$ when bond (9-10) eclipses the bond (3-4).

In the molecules with a side chain at C-2, the dihedral angle χ defines its orientation. $\chi = 0$ when the bond (X_1-X_2) eclipses the bond (1-2).

Energy Calculations

The total conformational energy of a molecule is expressed as

$$V_{tot} = V_{nb} + V_{es} + V_{\theta} + V_{tor}$$

where V_{nb} is the energy due to nonbonded interactions, V_{es} is the electrostatic interaction energy, V_{θ} is the energy due to bond-angle strain, and V_{tor} is the torsional potential energy.

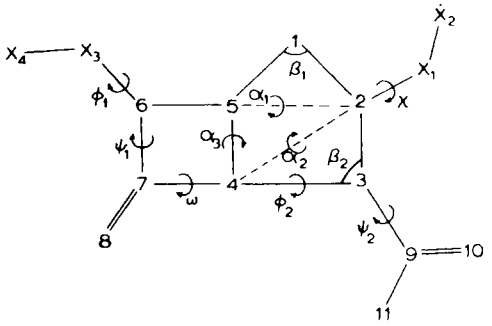

Fig. 1. Schematic representation of the numbering of atoms and dihedral angles.

The lactam peptide ring was fixed in a planar conformation as found in a number of crystal structures.[25–30] The atoms of the five-membered ring and the side groups were fixed with standard bond lengths and bond angles.[31] The energy of each molecule was calculated as a function of (α_1, α_2). These dihedral angles were varied in the range of −40° to +40° at 10° intervals and at each grid point the energy was minimized with respect to β_1, β_2, α_3, and the other conformational parameters which define the side groups.

For thienamycin nucleus (compound 1), the energy was minimized at each grid point with respect to β_1 β_2, α_3, and ψ_2. The favored value of ψ_2 was found to be ≈20°. Therefore, in all the related molecules, ψ_2 was fixed at 20° and the energy was minimized with respect to β_1, β_2, and α_3 only. Since in Δ^3-thienamycin, the C-3 carbon is Sp^3 hybridized, ψ_2 was fixed at 30°, the value found in simple peptides.[32] In the molecules which have

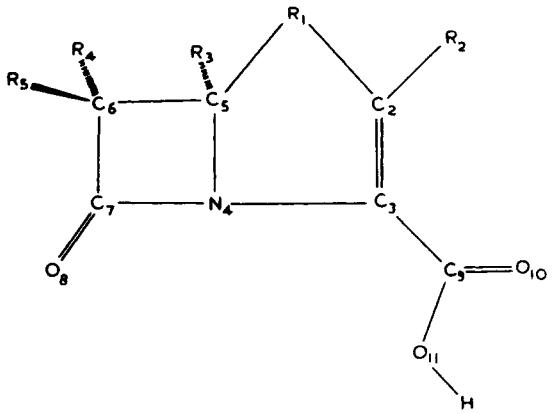

Fig. 2. Chemical structures of 1-carba, 1-thia-, and 1-oxa-β-lactam compounds: (1) R_1 = $-CH_2$, $R_2 = R_3 = R_4 = R_5$ = H; (2) $R_1 = -CH_2$, $R_2 = R_3 = R_5$ = H, $R_4 = -CHOH\text{-}CH_3$; (3) $R_1 = -CH_2$, $R_2 = -S\text{-}CH_2\text{-}CH_2\text{-}NH_2$, $R_3 = R_5$ = H, $R_4 = -CHOH\text{-}CH_3$; (4) $R_1 = -CH_2$, R_2 = $-S\text{-}CH_2\text{-}CH_2\text{-}NH_2$, $R_3 = R_4$ = H, $R_5 = -CHOH\text{-}CH_3$; (5) $R_1 = -CH_2$, $R_2 = -S\text{-}CH_2$-$CH_2\text{-}NHCOCH_3$, $R_3 = R_5$ = H, $R_4 = -CH_2\text{-}CH_3$; (6) $R_1 = -CH_2$, $R_2 = -S\text{-Ph}$, $R_3 = -CH_3$, $R_4 = R_5$ = H; (7) R_1 = S, $R_2 = -CH_3$, $R_3 = R_4 = R_5$ = H; (8) R_1 = O, $R_2 = -CH_2\text{-}CH_3$, R_3 = $R_4 = R_5$ = H; (13) 5S-isomer of compound 7.

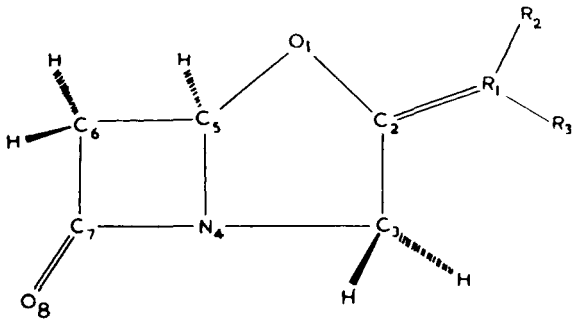

Fig. 3. Some analogs of clavulanic acid: (9) R_1 = C, R_2 = $-CH_3$, R_3 = H; and (10) R_1 = C, R_2 = H, R_3 = $-CH_3$.

a side chain at C-2, atoms beyond the sulfur atom were treated as a methyl group for computational simplicity. To start with, the dihedral angle χ was kept at 0°. Minimization was carried out with respect to β_1, β_2, α_3, and ϕ_1 for compounds 2–5 and 11. It was found that in compounds 2, 3, and 11, the favored values of ϕ_1 are around −172° and 80° and the energy difference between the conformations is very small (0.2 kcal/mol). For 6-epi-thienamycin and PS-5, the favored values of ϕ_1 are 170°, −80° and −170°, 88°, respectively. In the case of PS-5, the conformation with ϕ_1 = −170° has about 0.7 kcal/mol less energy than the other, and in 6-epi-thienamycin the conformation with ϕ_1 = −80° has about 0.3 kcal/mol less energy than the other. Then by keeping the bicyclic ring system in the favored conformation, the dihedral angle χ was varied to determine its favored values, i.e., are ≈46°, 86°, and −98°. Later ϕ_1 and χ were kept at

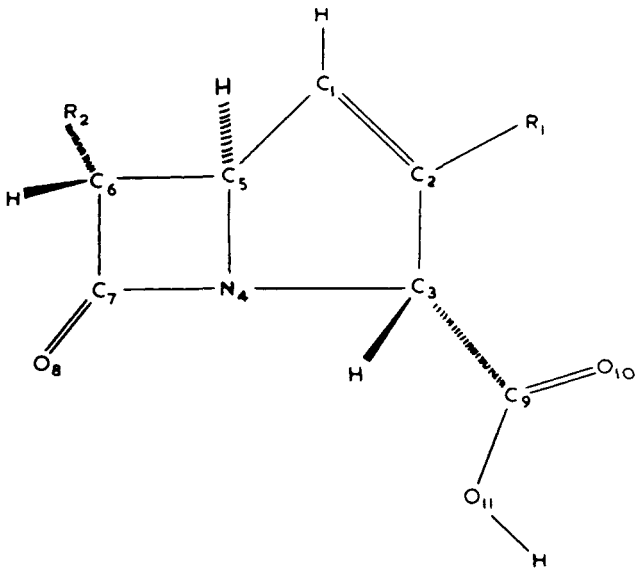

Fig. 4. Δ^3-Thienamycin (compound 11): R_1 = $-S-CH_2-CH_2-NH_2$, R_2 = $-CHOH-CH_3$.

each set of their favored values and the energy was minimized with respect to β_1, β_2, and α_3. However, it was found that the change in ϕ_1 and χ had an insignificant effect on the favored conformation of the bicyclic ring system. For compounds (6–10) also, minimization was carried out with respect to β_1, β_2, and α_3. Minimization was also done for the three favored values of χ of compound 6. Since it was found that the value of χ did not affect the conformation of the bicyclic ring system in compound 8, the R_2 group was treated as a methyl group. For compound 13, minimization was carried out with respect to β_1, β_2, α_3, and ψ_2.

In all these calculations, the methyl groups and the hydroxyl groups were treated as single united atoms with appropriate higher van der Waals' radii. The fractional charges on the various atoms were obtained as a sum of σ and π charges, computed following Del Re's method for σ-charges and Huckel's method for π charges.[33,34] The form of the functions, the parameters used to calculate V_{nb}, V_{es}, V_{θ}, and V_{tor} and the method of energy minimization are the same as reported earlier.[35] The isoenergy contours of these molecules on the (α_1, α_2) plane are shown in Figs. 5–7.

RESULTS AND DISCUSSION

Figures 5–7 show that in all molecules, α_1 and α_2 are highly restricted and a single minimum occurs around (5,−5). This is quite in contrast to penicillin, which favors two conformations (C_2 and C_3 puckered forms). Thus, the introduction of a double bond within or outside the five-membered ring at C-2 atom not only increases the rigidity of the molecule, but also leads to a single favored conformation. The favored values (Table I) of α_1, α_2, and α_3 also suggest that introduction of a double bond leads to a conformation between the C_2 (α_1, α_2 = 40°, 10°) and C_3 (α_1, α_2 = −10°,−25°) puckered conformations of penicillin.[36] Table I shows that in all these cases the lactam peptide bond is significantly more nonplanar ($\omega \approx 120°$) than in penicillins ($\omega \approx 135°$). The theory also predicts, in agreement with x-ray studies, that the lactam peptide bond in 1-thiapenem (compound 7) is less nonplanar than 1-carbapenem (compound 1).[10] Table I also suggests that the nature of the substituent and its configuration at the C-6 and/or C-2 atoms have a very minor effect on the favored conformation of the bicyclic ring system.

The relative orientation of the bicyclic rings ($180° - \alpha_3$) in compounds 1–11 is also different from that found in the C_2 (≈143°) or C_3 (≈113°) puckered forms of penicillin.[36] The carboxyl group favors a totally different orientation in compounds 1–8 ($\phi_2 \approx 75°$) from that found in penicillin ($\phi_2 \approx 161°$ or 111°). However, in compounds 11 and 12, the calculated values ($\phi_2 \approx 146°$) are close to those observed in the C_3 puckered conformation of penicillin. Thus, it seems that the introduction of a double bond in the five-membered ring of the antibiotic between the C-1 and C-2 atoms or between the C-2 atom and its substituent does not affect the orientation of the carboxyl group very much, whereas its introduction between the C-2

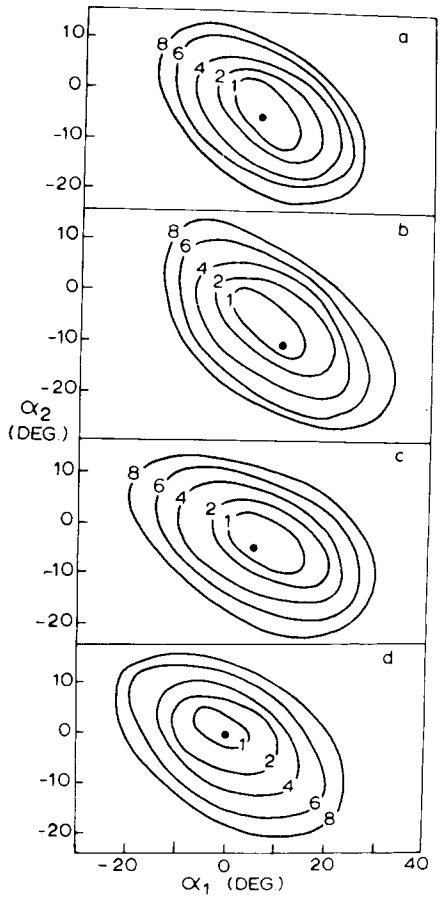

Fig. 5. Conformational energy maps of (a) thienamycin nucleus, (b) decysteaminyl thienamycin, (c) thienamycin, and (d) PS-5. Numbers on contours indicate energy (in kcal/mol). Position of the minimum is marked.

and C-3 atoms affects it significantly. The change in configuration at C-5 (compound 13), though it maintains the nonplanarity of the lactam peptide bond, has a drastic effect on the relative orientation of the rings ($180° - \alpha_3 \approx -126°$) and on the orientation of the carboxyl group ($\phi_2 \approx -75°$). Figure 7 shows that when the carboxyl group at C-3 is replaced by a hydrogen atom as in compounds 9 and 10, the five-membered ring becomes more flexible.

As mentioned earlier, few attempts have been made to explain the mechanism of action of β-lactam antibiotics at the molecular level. Lee[37] pointed out that the conformation of the substrate at its transition state is similar to that of the antibiotic. On the other hand, Rando[38] proposed that the induced strain on the antibiotics by the enzyme might be responsible for their activity. However, in both cases, to accommodate the substrate and the antibiotic, the same active site of the enzyme must be in-

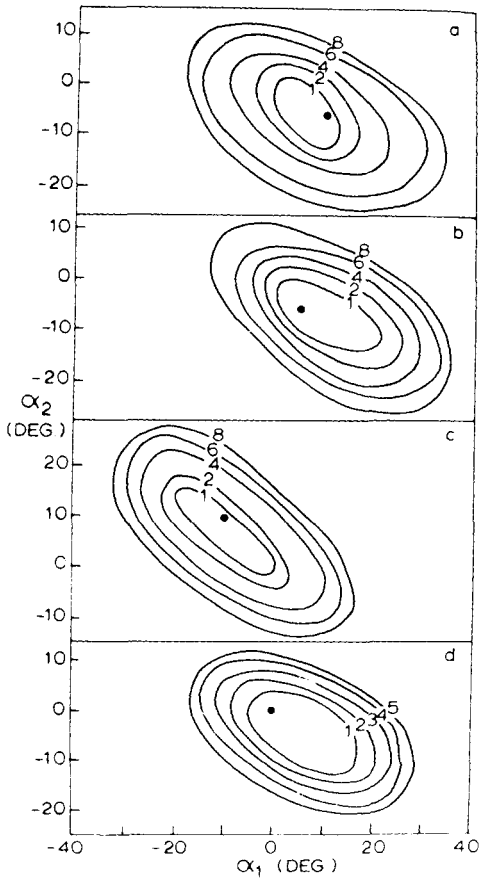

Fig. 6. Conformational energy maps of (a) compound 6, (b) 1-thiapenem, (c) 5-*S*-isomer of 1-thiapenem, and (d) compound 8. Numbers on contours indicate energy (in kcal/mol). Position of the minimum is marked.

volved. This suggests that the antibiotic should have the right conformation, or close to it, to bind with the enzyme.

It has been shown earlier[7] that β-lactam antibiotics with an L-configuration at C-6 atom are conformationally more similar to D-D peptides, whereas those with a D-configuration are more similar to L-D peptides, provided the configuration at C-3 is D. This explains the experimental observation that penicillin inhibits D-D-carboxypeptidases and transpeptidases more effectively than L-D-carboxypeptidases.[1,5]

It is interesting to note that the thienamycin nucleus (compound 1) exhibits broad-spectrum activity.[15] This shows that the conformation of the bicyclic ring system in this compound fits well into the active site of the crosslinking enzyme(s) involved in the biosynthesis of bacterial cell walls. Thienamycins and PS-5 (compounds 3–5) differ only in the nature and orientation of the substituent at C-6 atom. However, the substituents at

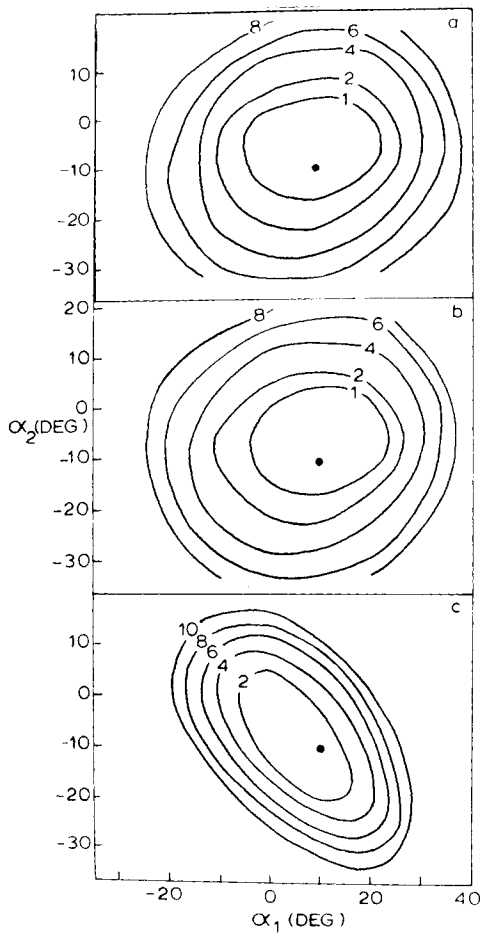

Fig. 7. Conformational energy maps of (a) compound 9, (b) compound 10, and (c) compound 11. Numbers on contours indicate energy (in kcal/mol). Position of the minimum is marked.

the C-6 atom in these cases are very small. It is interesting to note from Table I that the preferred values of α_1, α_2, α_3, ω, and ϕ_2 in these molecules are very close to that obtained in thienamycin nucleus. This is true for compound 2 also. This shows that the nature and orientation of the substituents at the C-6 or the C-2 atoms do not affect the conformation of the bicyclic ring system.

The fact that the 6-epi-thienamycin has an L-configuration at C-6 suggests that it can assume a conformation more similar to D-D peptides and, hence, would be highly active towards D-D-carboxypeptidases and transpeptidases. However, it has been shown experimentally[5] that thienamycin is also a reasonably good inhibitor of D-D-carboxypeptidases. This implies that the activity of the antibiotic is not drastically affected by the change in configuration at the C-6 atom, and it indicates that the

TABLE I
Calculated Conformational Parameters for Various β-Lactam Antibiotics in Their Minimum-Energy Conformations

β-Lactam Antibiotic and Compound No.	(α_1,α_2) (deg)	Non-planarity, ω (deg)	Orientation of carboxyl group, ϕ_2 (deg)	Relative Orientation of Bicyclic Rings, (180-α_3) (deg)	Activity[a]
1. Thienamycin nucleus	5, −5	119	76	121	A
2. Decysteaminyl thienamycin	10, −10	120	79	123	A$^+$
3. Thienamycin	5, −5	118	76	123	A$^+$
4. 6-epi, thienamycin	5, −5	120	74	124	A$^+$
5. PS-5	0, 0	117	75	124	A$^+$
6. —	10, −5	119	76	122	W
7. 1-Thiapenem	5, −5	128	73	126	A$^+$
8. —	0, 0	112	79	119	I
9. —	10, −10	123	—	121	I
10. —	10, −10	123	—	122	I
11. Δ^3-Thienamycin	10, −10	126	146	121	Useful as bactericide
12. Clavulanic acid[b]	10, −10	121	148	122	W, I
13. 5-*S*-Isomer of 1-thiapenem	−10, 10	−128	−75	−126	Inactive

[a] A, broad-spectrum, potent activity; A$^+$, broad-spectrum, potent activity including β-lactamase-producing strains; W, weakly active; I, inhibitor of β-lactamases.
[b] From Ref. 35.

enzyme could tolerate the change in configuration if the side group is small. The D-configuration at C-6 in thienamycin suggests that it should be highly active in inhibiting L-D-carboxypeptidases. In fact, experiments[5] have revealed that thienamycin is about 300 times more effective in inhibiting L-D carboxypeptidase than is benzylpenicillin. Recent experimental results[39] that 6-epi-thienamycin is antibacterially more active than thienamycin are in good agreement with the theoretical conclusion that the former would be more analogous to D-D peptides and would therefore be a better inhibitor of transpeptidases and D-D-carboxypeptidases.

1-Thiapenem (compound 7) has conformational parameters (α_1, α_2, α_3, and ϕ_2) similar to those of the thienamycins and PS-5. Thus, the theory predicts, in agreement with the experimental studies,[10-12] that it is biologically active. However, the slightly higher value of ω (less nonplanarity) suggests that this molecule may be slightly less active than the latter.

Table I shows that compound 6 also has conformational parameters similar to those of the other potent antibiotics in the new family of β-lactams. Such a conformational similarity should make this compound a very potent drug. Contrary to the expectations, this drug is only weakly active. On the other hand, when the methyl group at C-5 is replaced by a hydrogen

atom, though the favored conformation of the molecule is not affected, its activity increases significantly.[40] A projection of the compound in the minimum-energy conformation (Fig. 8) shows that the methyl group at C-5 is protruding outside the α-face of the molecule. Such an orientation of the group will interfere with the fitting of the antibiotic at the active site of the enzyme, if the enzyme approaches the β-lactam compound from the α-face.

Compound 13 is the 5-S isomer of compound 7. Such a change in configuration at C-5 does not affect the degree of nonplanarity of the lactam peptide bond, but it affects the other conformational parameters drastically. The favored conformation of compound 13 is the mirror image of that of compound 7. This brings in significant changes in the α- and β-faces of the molecule (Fig. 9), which explains its biological inactivity.[11,12] These results suggest that the configuration and nature of the group at C-5 is also important for biological activity.

The high activity of compounds 1–5 and 7 suggests that the favored conformation of the bicyclic ring system in these molecules is capable of preferentially binding in the active site of the crosslinking enzyme(s). If compounds 11 and 12 were also to bind in their preferred orientation in the same mode of binding as that of the compounds 1–5 and 7, the carboxyl groups of former may encounter some short contacts with the groups or atoms of the enzyme at the active site because of differences in their orientation ($\phi_2 \approx 146°$). Because of the highly rigid nature of the bicyclic ring systems, such contacts could be relieved either by a rotation of these drugs slightly out of the binding site or by the movement of some of the amino acid residues in the active site in order to accommodate the change in conformation of the carboxyl group of the drug, leading to a slightly different mode of binding. This may lead to a weak interaction, which in turn may result in lower activity.

It has been reported that compounds 3–5, 7–10, and 12 are good inhibitors

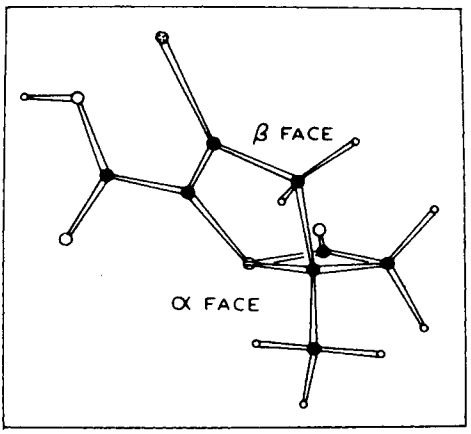

Fig. 8. A projection of compound 6 in the minimum-energy conformation. Symbols used: ⊖, sulfur; ●, carbon; O, oxygen; ⊖, nitrogen; and ∘, hydrogen.

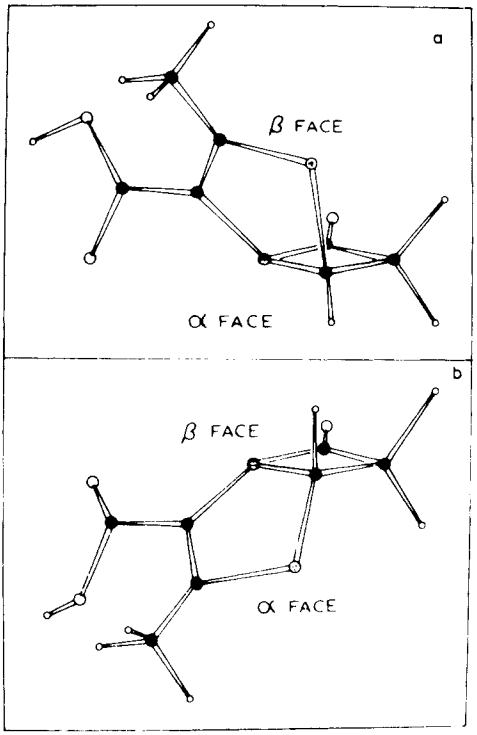

Fig. 9. Projections of (a) 1-thiapenem and (b) the 5-*S*-isomer of 1-thiapenem in their minimum-energy conformations. Symbols same as in Fig. 8.

of β-lactamases.[14,18,21,23,41,42] The orientation of the carboxyl group in compound 12 differs from that of other potent inhibitors. This suggests that β-lactamases are not highly specific for the orientation of carboxyl group. In compounds 9 and 10, which lack the carboxyl group at C-3, the bicyclic ring system has the same conformation as found in the other potent inhibitors. This suggests that the absence of the carboxyl group at the C-3 position does not affect the inhibitory properties significantly. Unlike its close analogs, thienamycin nucleus is shown to be less stable against β-lactamase-producing strains.[15] Since the bicyclic ring system has the same conformation in both thienamycin and thienamycin nucleus, the difference in the stability of these molecules against β-lactamase-producing strains suggests that the side groups play a dominant role in their stability.

Thus, the present study indicates that the conformation of the bicyclic ring system and the nature and orientation of the side groups are important in the inhibition of crosslinking enzyme(s) and in providing stability against β-lactamase-producing strains. Most of the drugs described here, which inhibit the biosynthesis of peptidoglycan, are also good inhibitors of penicillin β-lactamases. This suggests that the binding specificities of crosslinking enzyme(s) and penicillin β-lactamases are nearly identical as far as the bicyclic ring system is concerned.

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